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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference GRFBP6148381	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/02739	International filing date *(day/month/year)* 24.06.2003	Priority date *(day/month/year)* 24.06.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/68		
Applicant UNIVERSITY COURT OF THE UNIVERSITY OF ABE.. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.01.2004	18.08.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Moreno de Vega, C Telephone No. +49 89 2399-7486

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/02739

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-54 as originally filed

Claims, Numbers

1-40 received on 08.07.2004 with letter of 02.07.2004

Drawings, Sheets

1/29-29/29 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-40
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-40
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-40
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: HARAGUCHI, S. ET AL: "Immunosuppressive retroviral peptides: cAMP and cytokine patterns" IMMUNOLOGY TODAY, vol. 16, no. 12, 1995, page 595-603, XP001152891

D2: DUKERS, D. F. ET AL: "Quantitative immunohistochemical analysis of cytokine profiles in Epstein-Barr virus positive and negative cases of Hodgkin's disease" THE JOURNAL OF PATHOLOGY, vol. 190, no. 2, February 2000 (2000-02), pages 143-149, XP009018734

D3: ILAN Y ET AL: "Induction of oral tolerance in splenocyte recipients toward pretransplant antigens ameliorates chronic graft versus host disease in a murine model" BLOOD, W.B.SAUNDERS COMPAGNY, ORLANDO, FL, US, vol. 95, no. 11, 1 June 2000 (2000-06-01), pages 3613-3619, XP002165784 ISSN: 0006-4971

D4: DUKERS D F ET AL: "DIRECT IMMUNOSUPPRESSIVE EFFECTS OF EBV-ENCODED LATENT MEMBRANE PROTEIN 1" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 165, no. 2, 15 July 2000 (2000-07-15), pages 663-670, XP001145438 ISSN: 0022-1767

1. Document D1 teaches that retroviral envelope peptides cause a suppression of cell-mediated immunity by upregulation of interleukin 10 (IL-10).

   Document D2 discloses that EBV-positive cases of Hodgkin's disease (HD) contain a significantly higher percentage of neoplastic cells expressing the immunosuppressive cytokine IL-10 than EBV-negative cells.

   Document D3 teaches that serum IL-10 levels are higher in mice in which an oral tolerance towards pretransplant antigens has been induced.

   Document D4 teaches that EBV-LMP1 fragments have direct

immunosuppressive properties and that LMP1 is able to up-regulate the expression of IL-10 upon transfection into LMP1-negative Burkitt's lymphoma cell lines.

2. Article 33(2) PCT

Present claims 1-40 appear to be novel, as the known prior art does not disclose the present methods for tolerising a cell population to a target antigen (claims 1-7), the use of tolerogenic peptides of EBV LMP1 and LMP2 for the tolerisation of an individual against a target antigen (claims 8-14), the methods for assessing the tolerogenicity of a test peptide of claims 15-39 and the peptides of claim 40.

Claims 1-40 meet thus the requirements of Article 33(2) PCT.

3. Article 33(3) PCT

Claims 1-14 and 40 refer to methods and compositions for tolerising a cell population to a target antigen, whereby the cell population is contacted with a tolerogenic peptide sequence from EBV LMP1 or LMP2 protein and with the target antigen.

The present invention is based on the discovery that proteins from certain infectious agents, e.g. LMP1 and LMP2 from EBV, are tolerogenic in individuals previously infected by those infectious agents, i.e. the lymphocytes' responses to the target antigen remain suppressed, even in the subsequent absence of the tolerogenic substance, in which case the immune responses to other antigens are unaffected.

The prior art (see D4) shows that if LMP1 and an antigen or other stimulus are added to a population of lymphocytes, the reactivity of those lymphocytes to the target antigen is suppressed. The present invention shows that if LMP1 is then removed, those lymphocytes remain unable to respond to that target antigen, although they retain the ability to respond to other antigens.

This is not suggested by the prior art.

The present application shows that the peptides of claim 40 are capable of inducing immune tolerance to target antigens in individuals previously infected by EBV. This finding was not disclosed in the prior art.

Hence, claims 1-14 and 40 are considered to be inventive.

Present claims 15-25 and 34-39 are based on the discovery that testing the ability of a peptide from an infectious agent to induce IL-10 expression in lymphocytes from individuals previously infected by that agent provides a good indication of whether or not that peptide can be used to tolerise individuals previously infected with the infectious agent against other antigens. There is no hint in the known prior art to the methods of said claims.

Hence, claims 15-25 and 34-39 are considered to be inventive.

Present claims 26-33 refer to a method for assessing the tolerogenicity of a test peptide sequence from an infectious agent towards an antigen in a cell population, based on the determination of cell proliferation or expression of IL-4, IL-2, IL-12 or gamma-IFN by said cell population. The known prior art does not suggest the methods of said claims. Hence, claims 26-33 are considered to be inventive.

Therefore, claims 1-40 comply with the requirements as set forth in Article 33(3) PCT.